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{Exhibit 62}

"Diagnostic Immunology: Current and Future Trends," Cap Conference, Aspen, 1978, p. 67 and 80

CAP Conference on Diagnostic Immunology (1978)

DIAGNOSTIC IMMUNOLOGY:

Current and Future Trends

CAP CONFERENCE/ASPEN 1978

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Again after a suitable incubation period, the reaction is stopped, and the absorbance of the reaction product present in each well is determined. The amount of unknown antigen present is determined by subtracting the absorbance of the unknown from the absorbance of labeled antigen only and plotting the result against a standard curve. Table IV lists several applications of competitive binding EIA's.

Table IV

SELECTED APPLICATIONS OF COMPETITIVE BINDING EIAs

Antigen detected	Reference
IgG	Engvall and Perlmann, 1971
Alpha-fetoprotein	Belanger *et al*, 1973
Carcinoembryonic antigen	Engvall and Perlmann, 1975
Human chorionic gonadotropin	Van Weemen and Schuurs, 1971
Insulin	Kato *et al*, 1976
Thyroid stimulating hormone	Miyai *et al*, 1976
Cortisol	Ogihara *et al*, 1977
Gentamicin	Standefer and Saunders, 1978
Aflatoxin B_1	Lawellin *et al*, 1977

3. Operational Considerations

A. Choice of the Enzyme Amplifier

A list of enzymes used as amplifiers for heterogeneous EIA's is presented in Table V. The most commonly used enzymes have been alkaline phosphatase, β-galactosidase, and horseradish peroxidase (HRP). A single enzyme may not be suitable for every EIA performed at any single laboratory. Criteria used in choosing an enzyme amplifier for a particular assay include (1) turnover number (how fast does the enzyme convert substrate?); (2) sensitivity and safety of available substrate systems (certain chromogens used with HRP are carcinogenic, such as diaminobenzidene); (3) ability to couple the enzyme to the desired molecule without greatly altering enzyme activity; (4) stability of the conjugate produced; (5) availability and cost of the enzyme; (6) possible interference of test fluids with the enzyme system (for example, free hemoglobin may nonspecifically bind to the solid phase where it can catalyze substrates used in HRP EIA's); and (7) molecular weight of the conjugates obtained. This becomes especially important if the conjugate must penetrate cell membranes, as in indirect cell-bound virus EIA's [e.g., hog cholera (Saunders, 1977)].

Table V

ENZYME LABELS USED FOR SOLID-PHASE EIAs

Acetylcholinesterase	Glucoamylase
Alkaline phosphatase	Glucose oxidase
β-D-Galactosidase	Horseradish Peroxidase
Carbonic anhydrase	

B. Choice of Solid Phase

A variety of materials and configurations have been used as solid phase, some of which are listed in Table VI.

Table VI

SOLID PHASES USED IN EIAs

Cellulose acetate discs
Polystyrene and polyvinyl tubes, discs, beads, and microplates
Glass rods and beads
Tissue culture cells
Sepharose beads

Probably the most commonly used reaction vessel for manual testing is the polystyrene or polyvinyl microtitration tray as it is inexpensive, convenient to use, readily available and, most important, appears to work well for most assays. In the Technicon automated system to be described below, small polystyrene tubes are employed.

1. Introduction

Although enzyme immunoassays (EIA) are used for various purposes, this discussion deals only with the application of EIA to antibody detection. Although the procedure was originally designated ELISA (enzyme-linked immunosorbent assay) (Engvall and Perlmann, 1971), it now seems more appropriate to use the general term EIA. To date, no EIA standards have been provided upon which to base quality control. In some cases rather good previous serologic procedures can be used as guidelines, but actual quality control is not yet a functional reality.

Perhaps the first question to ask is what is a standard procedure? To some, this implies a procedure for which each step is explicitly described and must be followed exactly. However, even when well-defined procedures are meticulously performed, exact or reproducible answers are not guaranteed. To others, a standard procedure is simply one which produces the expected end product when tested against a standard.

Clearly, the ultimate goal is to obtain the reproducible correct answer. However, to paraphrase, "no lab is an island"; its personnel must continuously interact with the personnel of other laboratories. Consequently, specimens are submitted to more than one laboratory, reagents are exchanged, and, most importantly, reagent manufacturers must be able to provide satisfactory reagents to all. These factors dictate that a standard procedure be one which is completely and precisely described and performed without deviation in all laboratories. In order to attain such a goal, we must analyze all of the variables of the procedure in question and determine what modifications are needed. Unfortunately for our purpose, some variables such as the marker enzyme are subjectively rather than objectively selected.

2. Solid Substrates

A number of reports describe polystyrene as the most suitable solid substrate. Investigators have used microtitration plates (Voller *et al*, 1974), polystyrene tubes (Ruitenberg *et al*, 1974), latex-coated beads, paper discs (Halbert and Anken, 1977), Sephadex beads (Miranda *et al*, 1977), and polystyrene microcuvettes (Leinikii and Passilla, 1977). All of these have been successfully coated with antigen and used in EIA. We limited our investigation to microtitration plates and evaluated those offered by several manufacturers, including the special MicroELISA plate by Dynatech. We found that the applicability of these products varies with the antigen used. Dynatech (and Greiner Co.) markets a MicroELISA plate which purportedly is superior to all other microtitration plates.

When we used the toxoplasmosis system with horseradish peroxidase and ABTS as the chromogen and compared four microtitration plates [the MicroELISA, Cooke polystyrene (PS), Linbro polystyrene, and the Cooke polyvinyl chloride (PVC)], only minor variations in reactivity occurred. The designs of both the Cooke PS and the Linbro PS are flawed in that these plates have a glare around each well which causes some difficulty in reading. Also, the reactions are perhaps one well lower in these two plates than in the other two tested. The MicroELISA and Cooke PVC elicit comparable reactions, although the reactions in the PVC appear somewhat more intense. With the PVC, although slightly stronger reactions can be obtained, one deals with a flexible plate more difficult to handle. The rigid MicroELISA plate is easier to use but produces slightly weaker reactions and costs nearly three times as much as the PVC. Obviously, neither is the ideal plate.

Either plate can be read visually or with a microcuvette spectrophotometer.

For serodiagnostic purposes, visual readings have proven to be entirely satisfactory. Because of the inherent differences in antigens and the variation in adsorptive qualities of the support matrices, no standard plate can be recommended at this time.

3. Antigens

Antigens can and should be standardized. However, because of the magnitude of such an endeavor, this variable will probably be the last standardized. The antigens for each test must be individually compared and evaluated. One such evaluation to select a standard complement fixation antigen for Chagas' disease took the Pan American Health Organization five years and involved four laboratories before a standard could be established (Alemida, 1972). Because of the international impact, these sorely-needed evaluations should be carried out under the auspices of an international authority after priorities have been established.

4. Washing Methods

Washing represents perhaps one of the biggest and most easily solved problems encountered in the procedure. Simple flooding and shaking the diluent from the plates is inadequate unless extremely carefully done. The size and shape of each well allows droplets of diluent to be trapped and held or air bubbles to form and prevent the well from being filled. By delivering the diluent from a tube with a small orifice, one can direct the stream precisely into each well. Even despite such precautionary measures, air bubbles may form, and the wells may be incompletely washed.

Removing the diluent is even more difficult. Simply inverting and shaking the plate frequently does not affect the contents of some wells. This problem can usually be solved by vigorously rapping the plate on an absorbent towel placed on a flat surface. Of course, if one is working with an infectious antigen or with sera potentially contaminated with hepatitis or other agents, shaking the plates must be avoided. In such a case, one solution is to use a small-tipped suction tube, such as a Pasteur pipette, attached to a side-arm flask. The pipette can be rapidly and easily moved from well to well and the contents aspirated into a disinfectant solution.

Another method uses an instrument (Dynatech Corporation) which sequentially fills and aspirates all 12 wells in a row simultaneously. At each of the 12 positions is a delivery tip and an aspiration tip. Depressing the manifold activates the vacuum, which aspirates the solution into a side-arm flask that may contain disinfectant. Depressing the control lever introduces fresh diluent. The plate is then advanced and the process repeated. It should be noted that this instrument can only be used for washing and aspirating; measured amounts of diluent cannot be introduced. A model which can deliver measured amounts and is more convenient to use was displayed at the 1978 ASM national meeting but has not yet been marketed.

5. Control Sera

Perhaps the most likely standard reagent to be readily available is control serum. Several such products are currently available from WHO, and although few have been evaluated in terms of EIA activity, they have been well-characterized by other procedures and can readily be adapted to enzyme techniques. As is true with antigens, the variety of such products required for all tests makes selecting control sera a gigantic task, although it is probably the least problematic variable of those we